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Identification of a tetrahydroprotoberberine as a metabolite of trimetoquinol in the rat

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Evidence indicates that mammalian systems have the ability to synthesize tetrahydroprotoberberines (THPB) from 1-benzyltetrahydroisoquinolines (THIQ). Davis *et al.* [1, 2] have shown that tetrahydropapaveroline (THP), a THIQ, can be converted metabolically to THPB alkaloids in rat liver and brain preparations by an *S*-adenosylmethionine-dependent enzyme. N^5,N^{10}-Methylenetetrahydrofolate may also serve as a source of formaldehyde for forming the methylene bridge [3]. Furthermore, the THPB alkaloids have been identified as urinary products in rats after the administration of THP and in Parkinsonian patients receiving L-dopa therapy [4]. Kametani *et al.* [5] have also confirmed the formation of THPB from THIQ in rat liver and brain preparations. Numerous studies have shown that THPB alkaloids produce a variety of pharmacological effects, including sedation, tranquilization, analgesia, MAO inhibition, and inhibition of catecholamine uptake.

Trimetoquinol (TMQ) (**1**) (Fig. 1), a potent beta adrenergic agonist, may form a THPB metabolite, although such a metabolite has not been isolated from animals treated with TMQ. Moreover, would this metabolite contribute pharmacologically to the overall effect of TMQ? Therefore, the metabolism of TMQ was reinvestigated to search for the presence of a THPB alkaloid metabolite, and to examine its pharmacological activity. Saitoh *et al.* [6] have reported that the major route of metabolism for TMQ is the formation of 6-methoxy-TMQ (**2**) and 7-methoxy-TMQ (**3**), but no other metabolites. Our study describes the identification of a THPB alkaloid (**4**) and its methoxylated derivative (**5**) as metabolites of TMQ in rat urine and describes preliminary pharmacological effects of **4**.

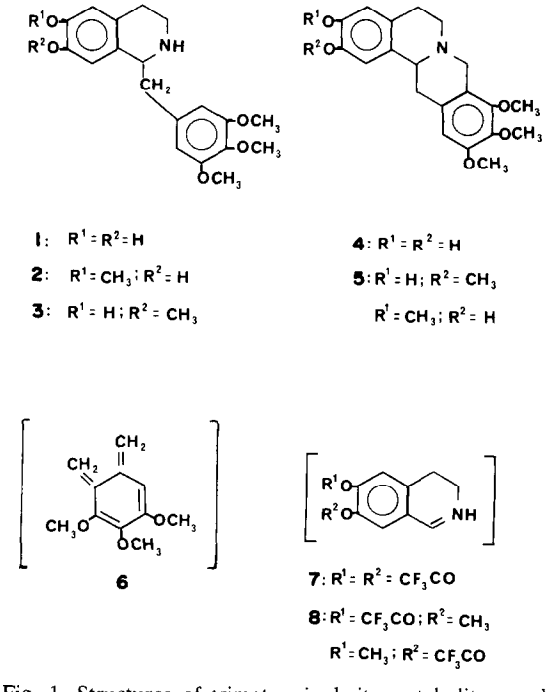

Fig. 1. Structures of trimetoquinol, its metabolites, and electron impact fragmentation ions.

Three rats were administered 50 mg/kg, i.p., of TMQ hydrochloride for 20 days in order to ensure the biotransformation of TMQ into THPB metabolites. On day 20, the 24-hr urine output was collected in a beaker containing 4 ml of 0.1 N hydrochloric acid. The urines were pooled and diluted to 70 ml with distilled water, to which was added 7 ml of 0.1 N hydrochloric acid. The urines were pooled and diluted to 70 ml with distilled water, to which was added 7 ml of 0.1 M sodium acetate buffer (pH 5) containing 35 mg ascorbic acid and 70 mg EDTA. Based upon the work of Saitoh *et al.* [6], TMQ and its methoxylated metabolites (**2** and **3**) are excreted as glucuronide conjugates (80%), with the remainder being unconjugated TMQ, **2** and **3**. Therefore, it was assumed that a similar situation existed in this study and no attempt was made to analyze for the unconjugated metabolites. A 35 ml portion of the urine was adjusted to pH 5.3 with dilute sodium hydroxide and incubated with 5 ml of Glusulase [β-glucuronidase (1000 units/ml) sulfatase, Endo Laboratories, Garden City, NY] at 37° for 8 hr. After this period, an additional 5 ml of Glusulase was added, and the incubation was continued overnight (18 hr). Then the mixture was adjusted to pH 7 and extracted several times with ethyl acetate. The ethyl acetate extracts were extracted with 0.1 N hydrochloric acid, and the acid extract was evaporated to dryness *in vacuo*. Control rats were administered an equivalent volume of normal saline i.p., and their urines were treated similarly. The dried residues were assayed with a Perkin–Elmer 3920B chromatograph using a 3% OV-1 column at 260° as their trifluoroacetates gave retention times (in secs) of 205 (TMQ), 325 (**2**), 350 (**3**), with new peaks at 420 (minor) and 640 (major), having relative areas of 1:2 respectively. The broad shape of the peak at 640 sec was nearly symmetrical, suggesting that it consisted of a single component. However, this does not preclude that two isomeric methoxylated THPBs had eluted together. The order and times of elution for TMQ, **2**, and **3** were similar to those reported by Saitoh *et al.* [6] and were confirmed by adding authentic TMQ, and a synthetic mixture of **2** and **3**, to an aliquot of the extract. Confirmation that the new metabolites were THPBs was provided by adding synthetic **4** to the urine extract. The chromatographic peak at 420 sec was increased in size while the peak at 640 sec was not, suggesting that is was the methyl ether of **4**. The THPB (**4**) was prepared by a Mannich-type reaction of TMQ with formaldehyde. The product was characterized as the HCl salt, m.p. 125–126°, elemental analysis for $C_{20}H_{23}NO_5 \cdot HCl \cdot 2.5\ H_2O$, and mass spectral analysis (*m/e*): 357 (M+), 194 ($C_{11}H_{14}O_3$, base peak), 179 ($C_{10}H_{11}O_3$), 164 ($C_9H_{10}NO_2$) and 162 ($C_9H_8NO_2$). The fragmentation pattern was similar to that found for other THPB alkaloids [7]. Two previously untreated rats were administered synthetic **4** (50 mg/kg, i.p.); the chromatogram showed similar peaks at 415 sec (minor peak) and 640 sec (major peak). Final proof of the identities of these new metabolites was obtained from gas chromatography–mass spectrometry data. The mass spectra for the THPB metabolites (as trifluoroacetates) were characterized by molecular ions for **4** at *m/e* 549, and for **5** at *m/e* 467. The fragmentation

pattern for **4** and **5** was dominated by cleavage of the trimethoxybenzyl group to give as the base peak the ion, **6** (*m/e* 194), and an isoquinolinium ion at *m/e* 356 (**7**), and 274 (**8**). The mass spectrum of **5** does not allow differentiation between the 2-OMe and 3-OMe positions.

The THPB alkaloid (**4**) was evaluated for its beta adrenergic activity using guinea pig tracheal chains that were stimulated with 10^{-5} M histamine. The results revealed that **4** (pD_2 5.61) was much less potent than TMQ (pD_2 7.85) or isoproterenol (pD_2 7.42) as a beta agonist. This is consistent with binding studies for THBPs on beta adenylate cyclase [8], which showed that THBPs were generally much weaker than their THIQ precursors. It may be inferred that the protoberberine structure is less preferred for binding, and that the trimethoxybenzyl moiety must be conformationally mobile for optimal binding to the beta receptor.

This study has demonstrated that TMQ can be cyclized to a THPB (**4**) in mammalian systems in low yields (<3% of total isolated metabolites), and that **4** apparently does not contribute significantly to the overall beta adrenergic activity for TMQ. Because the pharmacological results for metabolite **4** were not promising, the identification of metabolites **4** and **5** after a single dosing of TMQ was not completed.

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Use of vaccinia, a DNA virus, to study the role of DNA incorporation in the mechanism of action of 6-thioguanine*

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Previous results from this laboratory, using human tumor cells in culture [1] or a *Bacillus subtilis* transformation system [2, 3], have supported the theory [4] of incorporation of 6-TG† into DNA as one mechanism responsible for the biological activity of 6-TG. Other proposed mechanisms of action for 6-TG and the related compound, 6-mercaptopurine, include: (1) inhibition of *de novo* purine biosynthesis [5–7], (2) sequential blockade of guanine nucleotide biosynthesis [8], and (3) incorporation into RNA causing subsequent effects on RNA maturation [9–11] or translation [12]. The effects of these antitumor and immunosuppressive agents have been reviewed [13].

In mammalian cell systems, it is often difficult to determine which biochemical events are causally related to 6-TG treatment because of marked drug cytotoxicity. A DNA virus, vaccinia, was used in this study as a model to investigate possible alterations in DNA function which occur as a consequence of 6-TG incorporation. This virus replicates in the host cell cytoplasm; therefore, effects of the drug on viral genome expression can occur independently of that of the host cell. A preliminary report of this work has been presented [14].

Methods

Cell and virus cultures. HeLa and Vero cells, originally obtained from Flow Laboratories (Bethesda, MD), were continuously passaged in RPMI 1640 or McCoy's 5a media (Gibco, Grand Island, NY) supplemented with FCS (K.C. Biologicals, Lenexa, KS) and antibiotics (0.25 μg/ml amphotericin and 100 units/ml of penicillin and streptomycin). Vaccinia virus (American Type Culture Collection, Rockville, MD) was grown and passaged in HeLa cells. Experiments were performed in a variety of flasks and

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† Abbreviations: 6-TG, 6-thioguanine; FCS, fetal calf serum; PBS, phosphate-buffered saline, 0.14 M NaCl plus 0.01 phosphate buffer, pH 7.4; and PFU, plaque forming units.